Development of an Autonomous Ammonium Fluorescence Sensor (AAFS) with a View Towards *In-situ* Application

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Award Number: *N000141010210*

LONG-TERM GOALS

Our goal is to develop a portable autonomous ammonium sensor. Such a sensor could be deployed for periods of up to a month aboard ships, moorings or drifting buoys or used as a component in lowered or towed oceanographic instrument packages for vertical profiling.

OBJECTIVES

Our technical objective is to develop a robust, relatively simple, inexpensive, low power and compact instrument with a detection limit in the nM range and a sampling frequency of at least 4 samples per hour. Robustness, simplicity, low construction cost, lower power and small size are the practical desiderata for commercial application. Commercialization and the lowered instrument costs that will result are essential to permit wider application throughout the oceanographic community.

APPROACH AND WORK PLAN

Our approach has been to first design, assemble and demonstrate engineering prototypes suitable for bench-top laboratory use, take these aboard ship and once we have achieved the key design objectives test these first in an ongoing sampling program at coastal sewage outfalls along the east coast of Florida and second underwater by taking advantage of in-situ installations such as the “permanent” large mooring associated with the NOAA underwater habitat in the Florida Keys. Once

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **2013**	2. REPORT TYPE	3. DATES COVERED **00-00-2013 to 00-00-2013**
4. TITLE AND SUBTITLE **Development of an Autonomous Ammonium Fluorescence Sensor (AAFS) with a View Towards In-situ Application**		5a. CONTRACT NUMBER 5b. GRANT NUMBER 5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)		5d. PROJECT NUMBER 5e. TASK NUMBER 5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **University of Miami,Rosenstiel School of Marine and Atmospheric Science (RSMAS)/CIMAS,4600 Rickenbacker Causeway,Miami,FL,33149**		8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)		10. SPONSOR/MONITOR'S ACRONYM(S) 11. SPONSOR/MONITOR'S REPORT NUMBER(S)
12. DISTRIBUTION/AVAILABILITY STATEMENT **Approved for public release; distribution unlimited**		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT		
15. SUBJECT TERMS		

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**10**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

we have achieved (and verified in the field) all our basic design objectives we will then concentrate upon further miniaturizing, reducing power consumption as much as possible and re-packaging for the possible alternative applications. These last efforts will be facilitated by the large and diverse South Florida user community and the related instrument development activities occurring both at UM/CIMAS and AOML. Key personnel in the initial steps are Dr. Amornthammarong, Dr.Ortner, Dr. Hendee, Dr. Leonardi and Mr. Shailer Cummings, while assisting in these initial steps, will take a much larger role with regard to underwater deployment and field testing.

WORK COMPLETED

Since the project started, progress has been rapid and specific advances made with regard to the basic mechanics of instrumentation. First an effective, simple mixing chamber was designed that could be used in conjunction with a syringe pump for flow analysis. Second a new design of fluidic system was developed incorporating this mixing chamber, the Autonomous Batch Analyzer (ABA) system, and the ABA was repeatedly tested in the field (on a ship, at a coastal inlet, etc.). Both have been described in the peer-reviewed literature (Amornthammarong et al, 2010 and 2011). Building upon these advances, a submersible, battery-powered system is being constructed (Figure 1). The electrical port provides access to data logger, communication and battery modules. This will either be self-contained and submersible or be incorporated into larger integrated buoy or moored systems in order to facilitate longer deployment periods and take advantage of pre-existing data communication channels.

Figure 1. Submersible, battery-powered system for *in-situ* ammonium measurement in natural waters (currently in final assembly).

RESULTS

A simple, effective mixing chamber used in conjunction with a syringe pump for flow analysis was developed and thoroughly evaluated in the laboratory. It was constructed using a conventional 5 ml pipette tip and its performance compared with a widely-used mixing coil. The results obtained demonstrated that the standard mixing coil does not rapidly and completely mix solutions. Utilizing a configuration that reversed solution positions in the chamber with each mixing cycle, the proposed mixing chamber was able to achieve complete mixing in a significantly shorter time than the mixing

coil. The influence of injected sample volume on absorbance signals was then evaluated by calculating an $S_{1/2}$ value for the system. As tested with a minimal rinse, the system has no discernable carryover. Testing this new approach in our previously described silicate measurement system resulted in a more than two fold improvement in sensitivity (see Amornthammarong et al, 2010).

An autonomous batch analyzer (ABA) was then developed for the measurement of ammonium in natural waters. The system combined previously described batch analysis and continuous flow analysis methods and our new mixing chamber (see Amornthammarong et al, 2011). With its simpler design, the ABA system is robust, flexible, inexpensive, and requires minimal maintenance. The sampling frequency is ca. 8/hr and the potential limit of detection ca 1 nM which is comparable to the most sensitive flow through or batch analysis methods previously described and within the design specifications we had set for our project. In addition, the system produces a calibration curve by auto-dilution from a single ammonium stock standard solution with the same accuracy as traditional manual calibration methods. This last aspect is particularly important for extended (one month or longer) *in-situ* deployments.

Representative results obtained during the field tests are given below. Figure 2 plots underway measurements of ammonium in the surface seawater in South Florida coastal waters, including Florida Bay, Florida Key and Southwest Florida shelf waters taken aboard a cruise of the UNOLS vessel, RV/F.G. Walton Smith. Concentrations were elevated at several near shore locations near freshwater outflows from the Big Cypress/Southern Everglades. The shipboard system was remotely controlled and monitored from Miami over the Internet without any operating technician on board. It was then deployed at shore-locations. Figure 3 shows the ammonium concentrations in the intracoastal waterway South of Lake Mabel (Port Everglades, Fort Lauderdale, FL) during May 17-20, 2011. The results clearly show a cycle with ca. 24-25 hr periodicity. Figure 4 shows the ammonium concentrations in the Port Everglades inlet from June 30 – July 6, 2011. There were two ammonium maxima around 4 am and 4 pm every day from 1-3 July. The ammonium cycles closely match the tidal cycles in the inlet with outflow water carrying high ammonium concentration. The ammonium concentrations were low during holidays (3-4 July) and a heavy rainfall day (5 July). The data were much more variable than those from the intracoastal waterway South of Lake Mabel. Then, the instrument was deployed again at the Port Everglades inlet immediately after a heavy rain prior to the deployment (from October 16-20, 2011). Overall much higher concentrations of ammonium (3 μM) were observed early in the deployment. Maximum ammonium concentrations decreased from 3 to 1 μM in 4 days and continued to decrease to 0.1 μM as shown in Figure 5. The instrument was then deployed at the pond under the AOML/NOAA building. The results (Figure 6) clearly show diurnal cycles of ammonium from May 26 to June 19, 2012. In contrast to the results obtained in the intercoastal waterway and in coastal waters, ammonium concentrations in this tidal pond were high during the day time and dropped to below detection during the night. We have at present no definitive explanation for these differences. They are the subject of continuing research which will include simultaneous measurement of relevant uptake and generation processes.

Figure 2. Surface ammonium concentrations in Florida Bay and vicinity during June 7-11, 2011.

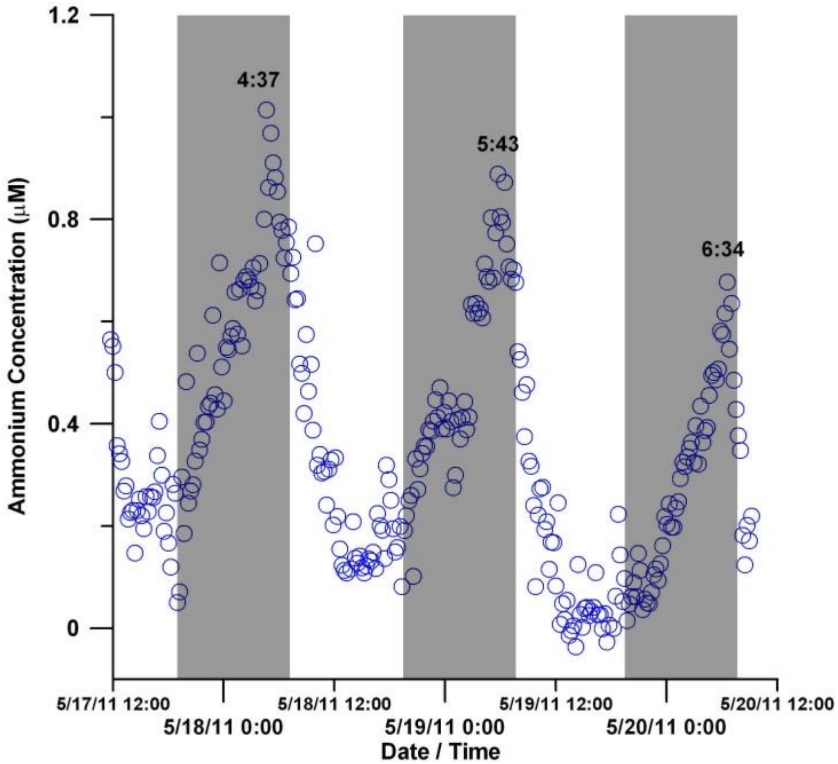

Figure 3. Measurements of ammonium in the surface waters of the intracoastal waterway South of Lake Mable from May 17 to May 20, 2011.

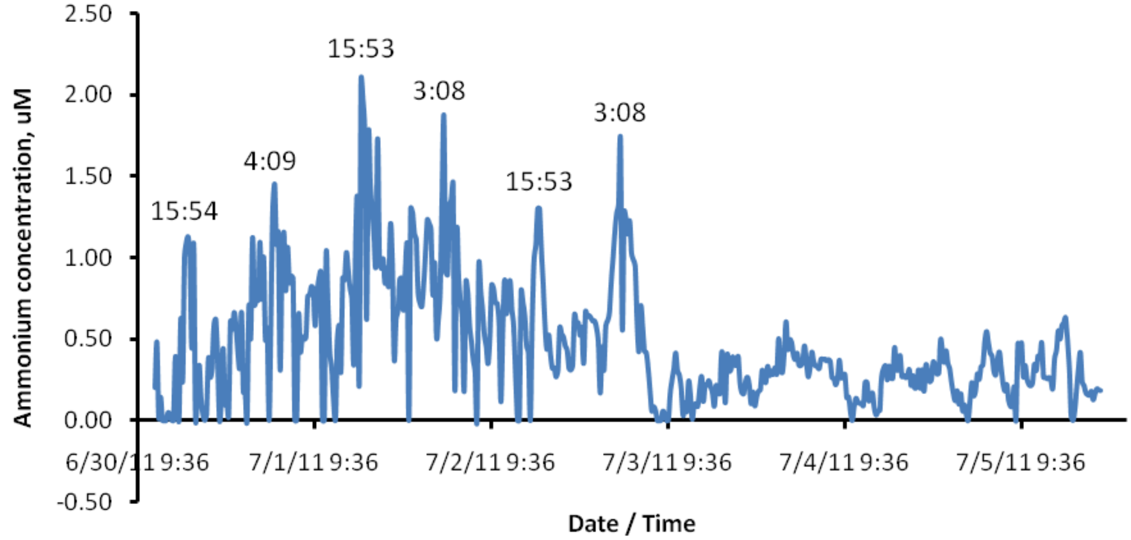

Figure 4. Ammonium concentrations at Port Everglades inlet, Fort Lauderdale, FL from 6/30/11 to 7/6/11.

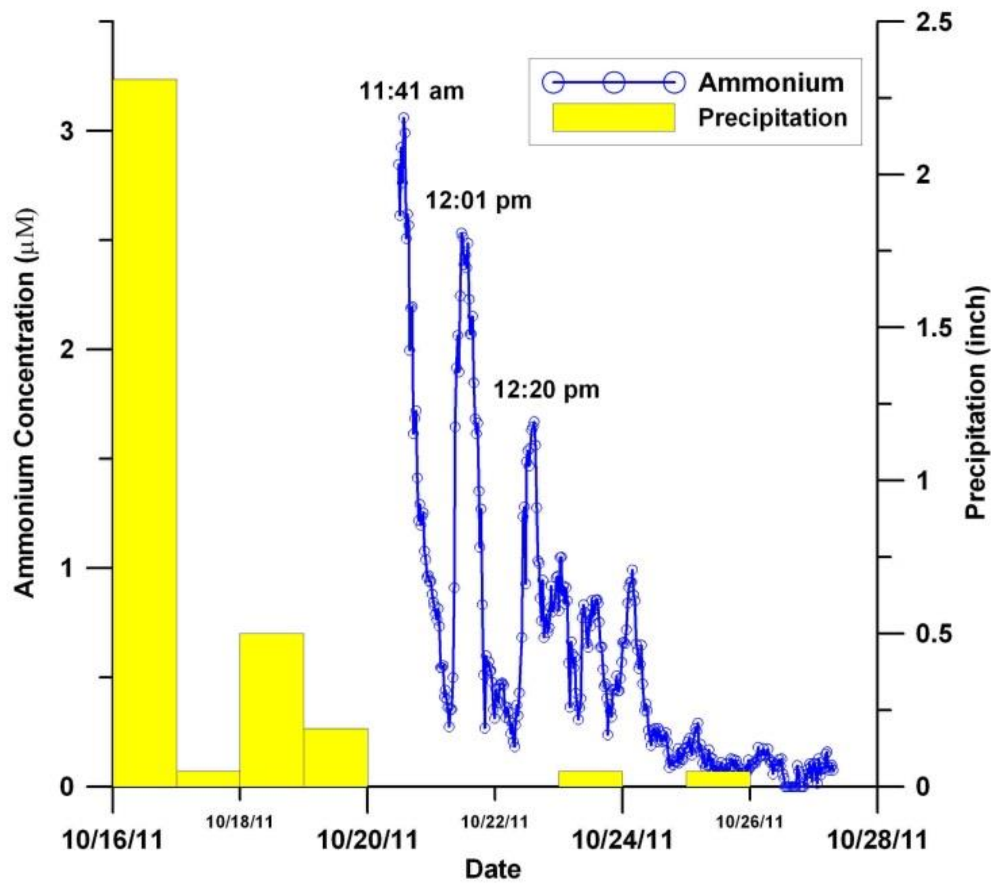

Figure 5.
Measurements of ammonium in the surface waters at Port Everglades inlet, Florida from October 20 to October 28, 2011.

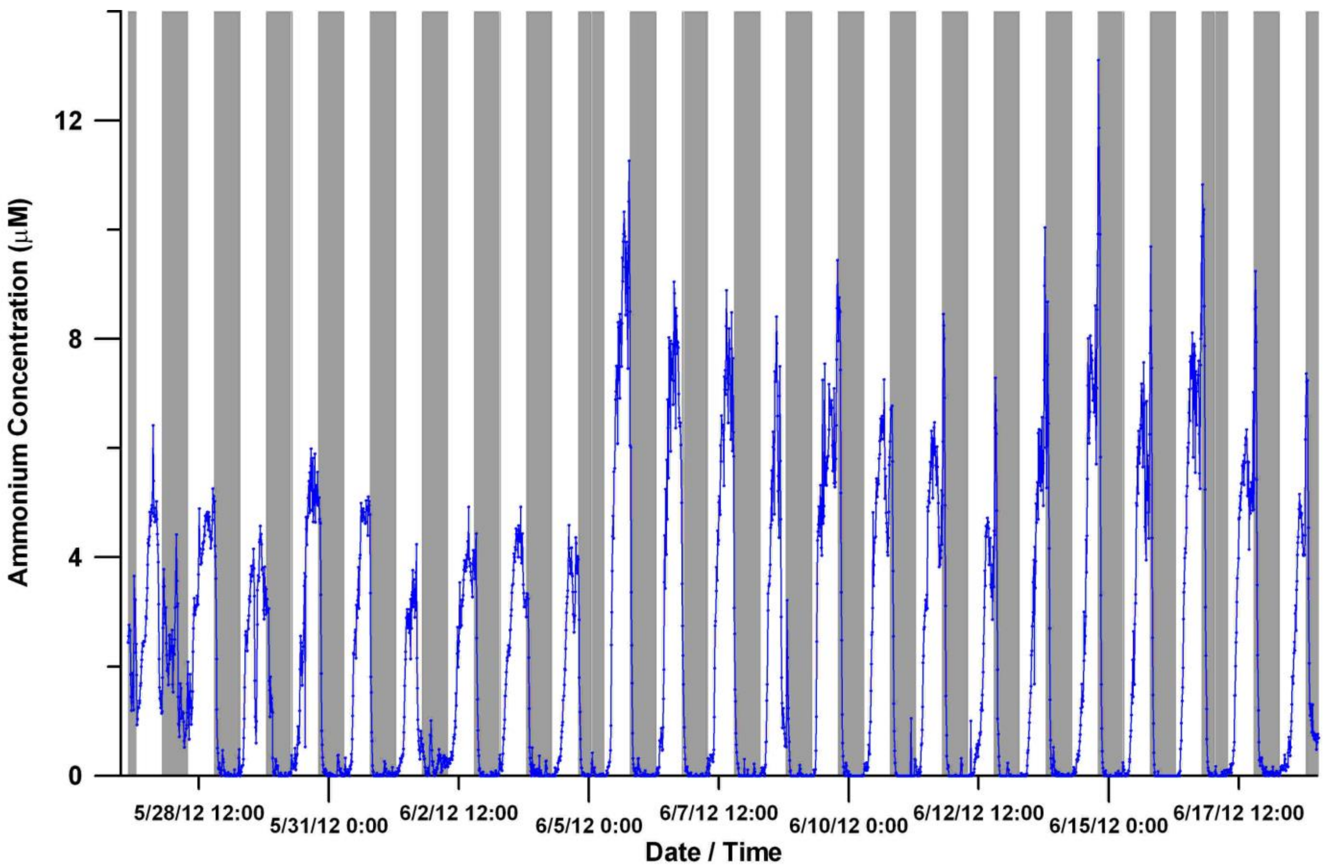

Figure 6. Measurement of ammonium concentration in the surface waters of the pond under the AOML/NOAA building from May 26 to June 19, 2012.

The system was also deployed for 20 days from September 23 to October 12, 2013 near a Land/Ocean Biogeochemical Observatory (LOBO) station at Harbor Branch Oceanographic Institute (HBOI), Florida Atlantic University for near real-time ammonium concentration measurement every 30 minutes in the Indian River Lagoon (IRL). From past 2 years IRL-monitoring of HBOI, ammonium dominates the DIN pool in the lagoon. High ammonium concentrations in tributaries and tidal creeks in residential areas with high densities of septic tanks indicate that much of the ammonium is from septic tank effluent. This is supported by stable nitrogen isotope data that shows highly enriched values characteristic of sewage nitrogen in groundwater, surface water and algal tissue. The entire results of the operation plotted with daily precipitation are shown in Figure 7. Overall much higher concentrations of ammonium were observed immediately right after a heavy rain prior to the deployment. In the first four days period a strong diurnal pattern was apparent. The ammonium concentrations of the period are plotted with chlorophyll data retrieved from the LOBO station (Figure 8). The result shows that ammonium concentrations were low when chlorophyll concentrations were high and vice versa.

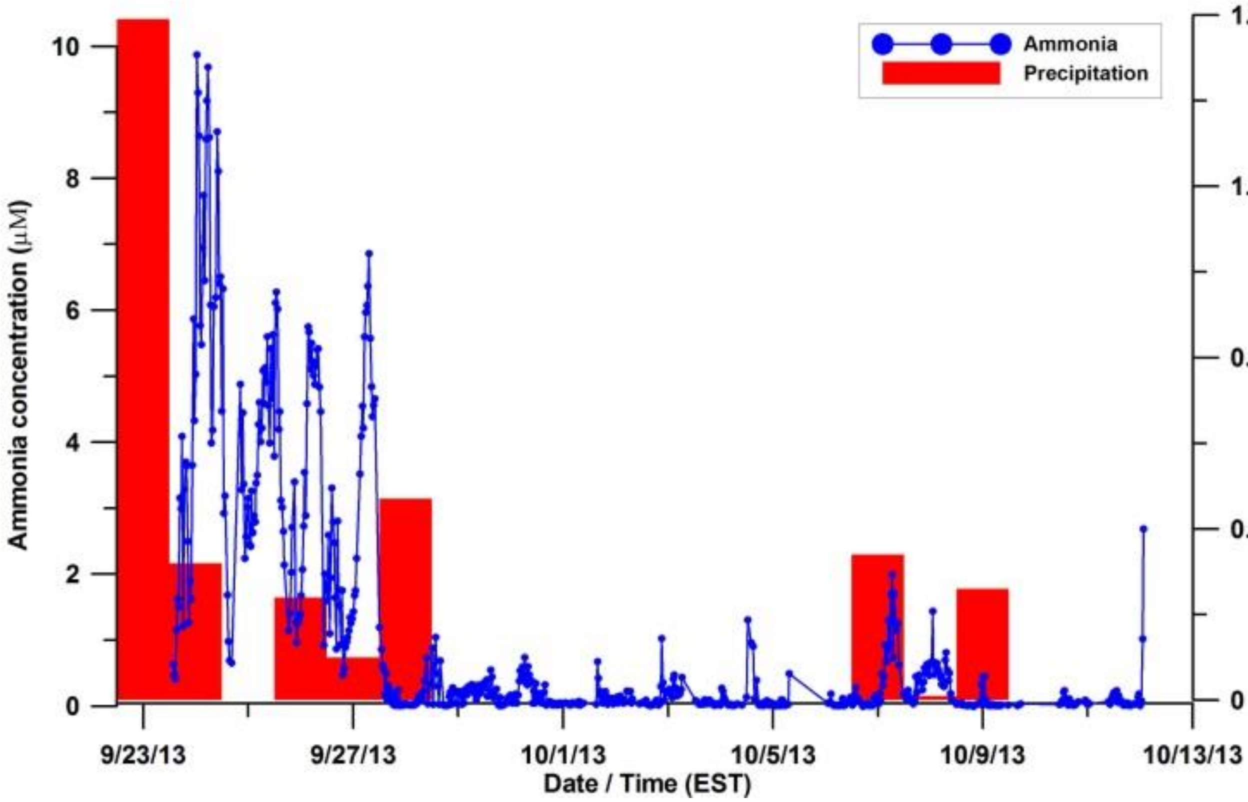

Figure 7. Measurements of ammonium in the surface waters of the Indian River Lagoon, Florida, at the pier of the Harbor Branch Oceanographic Institute, Florida Atlantic University from September 23 to October 12, 2013 (plotted with daily precipitation).

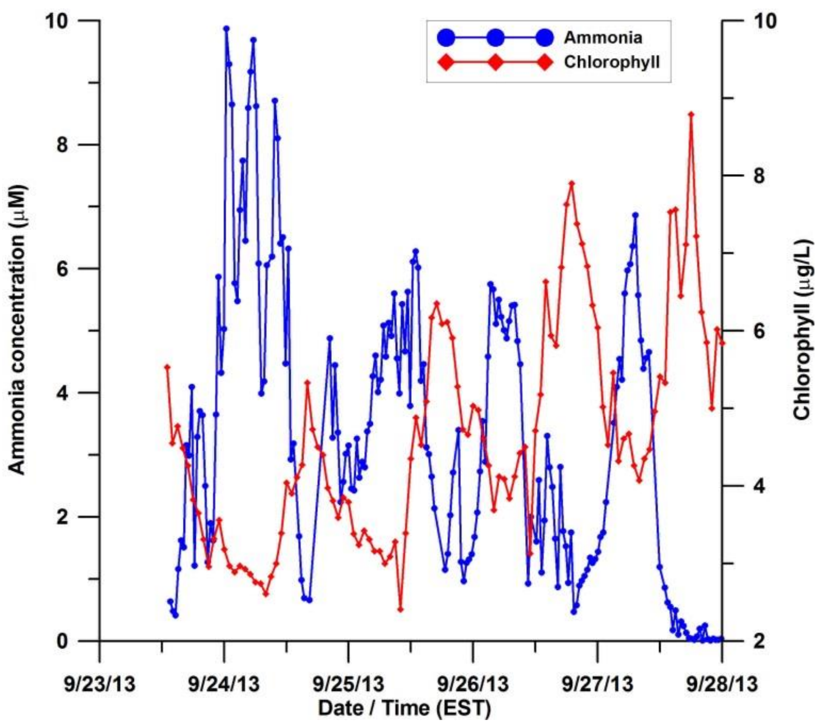

Figure 8. Effect of ammonium concentrations on chlorophyll concentrations.

When the ammonium concentrations of the first four days period are plotted with current direction as shown in Figure 9, it suggests that the source of the ammonium was north of the measurement point. Moreover, when the ammonium concentrations are plotted with conductivity (Figure 10), the result shows that ammonium concentrations were high when conductivity was low and vice versa. It suggests that ammonium may come from freshwaters.

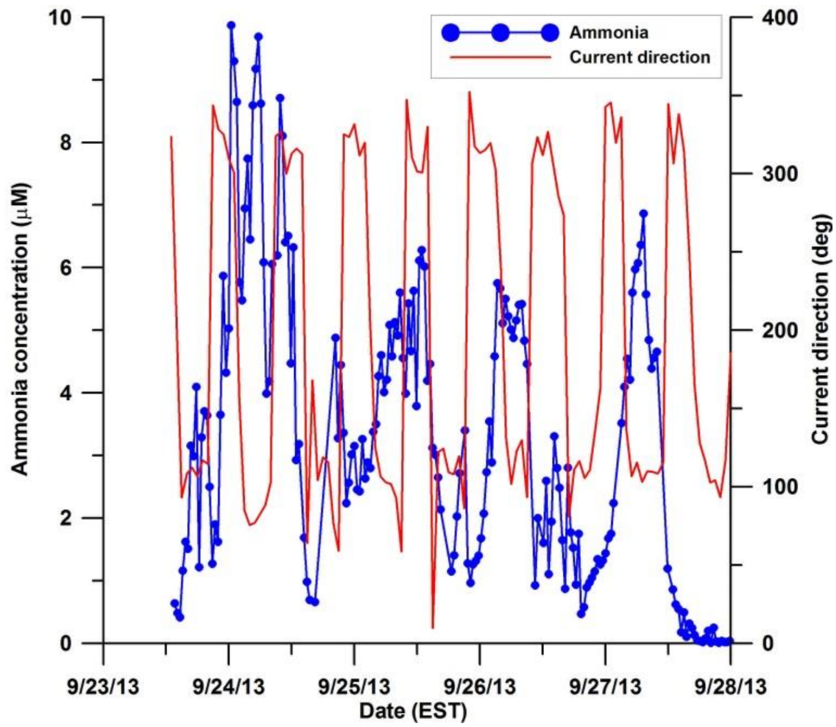

Figure 9. Ammonium concentrations are plotted with current direction.

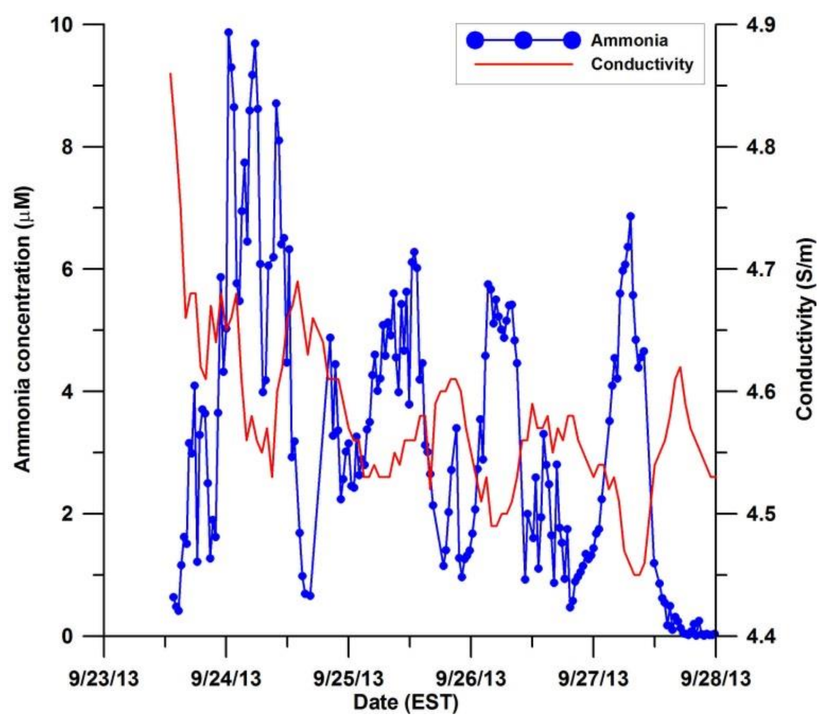

Figure 10. Ammonium concentrations are plotted with conductivity.

IMPACT AND APPLICATIONS

Economic Development

The sensor system being developed will have broad applicability as a research tool in biological oceanography but with respect to economic development it also has wide market potential for regulatory-required monitoring of ammonium. Such monitoring has become part of the permitting process for municipal and regional waste water treatment facilities throughout the U.S. Moreover, the basic design we have pioneered through the ABA system can be adapted to automating other wet chemical reactions such as nitrate, nitrite and phosphate, etc. further extending the commercialization potential of this NOPP-supported research and development project.

Quality of Life

Given the central ecological significance of ammonium in coastal and oceanic ecosystems a sensor system permitting long-term and near real-time cost effective measurements will be of significant assistance with regard to ecosystem based management of coastal living marine resources. Ecosystem based management is becoming a requirement of federal resource management and this implies measurement of key nutrients on the time and space scales relevant to ecological processes and changing ecosystem function.

Science Education and Communication

With respect to science education the primary relevance will be incorporation of the system (and the measurements it permits) in graduate theses and dissertations within the marine science community. Moreover data streams from contexts of local political significance (e.g. documenting the extent of pollution associated with individual point sources like sewage outflows or groundwater springs could be useful for public outreach and education.

When the development and testing is complete we will be able to deploy such an instrument to monitor in situ ammonium in the coastal and ocean water column to study the variable influx of this rapidly assimilated nutrient that is associated with migration of zooplankton populations in benthic communities (including coral reefs), zooplankton and mesopelagic fish vertical migration, grazing by schooling herbivorous fishes and intermittent physical processes such as breaking internal waves, wind-mixing etc.

TRANSITIONS

Economic Development

Contacts have already been established (and interest expressed) by commercial instrument manufacturers.

Quality of Life

The instrument has already been used in the Florida Area Coastal Environment (a federal/state/private industry partnership) to monitor surface concentrations of ammonium in the coastal waters of the Florida Keys and south-eastern coastal waters with respect to point sources like inlets adjacent to population centers and sewage outfalls.

RELATED PROJECTS

NONE

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